Part #15

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re: Anagnostou et al. Confirmation No. 9917
Serial No.: 09/525,797 Examiner: S. Ungar
Filed: March 15, 2000 Group Art Unit: 1642
For: *METHOD OF TREATING ENDOTHELIAL INJURY*

July 15, 2003

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R § 1.132
OF GEORGE SIGOUNAS, Ph.D.

Sir:

I, George Sigounas, Ph.D., do hereby declare and say as follows:

1. I received my Ph.D. from Boston University in Cellular Biology. I am currently Professor of Medicine at East Carolina University School of Medicine in Greenville, North Carolina. I am a co-inventor on the above-identified patent application.

2. The following experiments were carried out under my direction. These experiments were designed to test the effects of administering erythropoietin in conjunction with cisplatin. In particular, the present study is directed toward investigating the effects of simultaneous administration of erythropoietin and cisplatin on tumor weight.

3. To perform the *in vivo* studies, we employed the following protocol.

Animals

Female C57BL/6 mice, 7-8 months old and obtained from either Harlan or Jackson Laboratories, were used in this study. Mice were housed in groups of 4-8 in plastic cages and allowed ad libitum access to mouse food and water.

Injections

Single cell suspensions of LLCC (2 X 10^6/0.1 ml/mouse, >85% viability) were injected subcutaneously into the right front leg axillary. These cells form highly vascularized tumors. The day of cell implantation was designated day 0. After cell injection, tumor appearance, tumor growth, and tumor volume were assessed daily. Erythropoietin (EPO) and cisplatin (Cis) were administered intraperitoneally (i.p.). Unless stated otherwise, EPO and Cis were used at concentrations of 60 u/kg and 5-10 mg/kg, respectively. In each experiment, groups of 6-16 mice were treated with each regimen and schedule. Weight of the animals was monitored at least two times per week, and the physical activity of the mice was observed daily.

Groups: Control, injected with PBS (saline) alone
Epo, injected with erythropoietin alone
Cis, injected with cisplatin alone
Epo/Cis, injected with erythropoietin and cisplatin at the same time

Injections: two times per week (every Tuesday and Friday) during the study

Thirteen days following LLCC injection, animals were euthanized with a high dosage of anesthetic. The primary tumors were separated from the surrounding muscles and dermis, excised, weighed, and fixed in formalin for histological analysis.

4. The graph shows the results from three experiments. Tumors were removed 12-13 days following the initial injection of cancerous cells. The weight of tumors obtained from animals injected with Epo and Cis simultaneously was reduced by as much as 34% compared to animals injected with Cis alone. This difference was statistically significant ($P<0.05$).

Our studies indicate that administering erythropoietin in conjunction with cisplatin can reduce tumor size, and thus, can be used to treat solid vascularized tumors.

5. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

George Sigounas

George Sigounas, Ph.D.

7-14-03

Date

Group	Mean	SE
Control	1772	117
Epo	1566	115
Cisplatin	688	61
Cis/Epo	453	82